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ORIGINAL PAPER

Clear felling and burning effects on soil nitrogen transforming bacteria and actinomycetes population in Chittagong University campus, Bangladesh

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Abstract: The effect of forests clear felling and associated burning on the population of soil nitrogen transforming bacteria and actinomycetes are reported at three pair sites of Chittagong University campus, Bangladesh in monsoon tropical climate. Clear felled area or burnt site and 15−21 year mixed plantation of native and exotic species, situated side by side on low hill having Typic Dystrochrepts soil was represented at each pair site. At all the three pair sites, clear felled area or burnt site showed very significantly ($p \leq 0.001$) lower population of actinomycetes, *Rhizobium, Nitrosomonas*, *Nitrobacter* and ammonifying as well as denitrifying bacteria compared to their adjacent mixed plantation. From environmental consideration, this finding has implication in managing natural ecosystem.

Keywords: Nitrogen transforming bacteria; actinomycetes; forest clear felling effects; burning effects; soil microorganisms; Chittagong Hill Tracts

Introduction

In Bangladesh, clear felling of forests followed by artificial regeneration silvicultural system started with the introduction of *Tectona grandis* (teak) from the neighboring country Myanmar, then Burma, and in 1871 replacing natural forest at Kaptai of Chittagong Hill Tracts (CHTs). With the initial success of introducing this most valuable and high quality timber species, clear felling silvicultural system is adopted for this species and several other timber trees in the country, now. In this silvicultural system, all previous aged trees are harvested first, and then all the remaining vegetation on land consisting of small trees, shrubs and herbs are cut, dried and burnt sequentially to prepare the land for planting seedlings. This system brings temporal to complete change in soil and climate, and the changes become even greater when burning is conducted after clear felling. Burning reduces content and depth of organic matter, and decreases total nutrient pool of forest floor (Rosoman 1994; Certini 2005). Severe fire alters above and below ground species composition, generates volatilization of nutrients, degrades soil physical properties, decreases macro fauna, increases nitrogen load and sediment yield in stream water, changes in soil hydrologic functioning, affects greatly microbial population, variety and associated processes (Deka and Mishra 1983; Giovannini et al. 1988; Arocena and Opio 2003).

Management of nitrogen cycle has ecological, financial and environmental impact. World's ecosystem is influenced in either excess or deficiency of nitrogen. Among many different microbial mediated processes in the global nitrogen cycle, ammonification, nitrification, denitrification and nitrogen fixation are very much important in maintaining soil fertility and natural ecosystem. Biochemical release of ammonium from fresh import of organic matter remains in soil for short time and transformed rapidly to other N-pools in the soil and plant system. Transformations to various other pools of nitrogen also occur through volatilization, nitrification, plant uptake, microbial immobilization, ion exchange and formation of organic matter complexes (Killham 1994). The ecological significance of nitrification is that this process supplies nitrogen to the plant in its most available form, NH_4^+ and NO_3^-. Nitrate anion is readily leached through the soil profile into drainage water. Leaching of nitrate nitrogen is up to 100 $kg \cdot ha^{-1} \cdot a^{-1}$ from arable land in UK (Killham 1994) and much higher in Bangladesh situated in tropical countries due to high rainfall and application of high amount of nitrogenous fertilizer, representing a considerable economic loss as

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well as being of environmental concern. One adverse environmental consequence of high nitrite leaching is eutrophication, which decreases oxygen in water and occurs in the subsequent kills of aquatic animals including fish. In addition, nitrate is considered to be hazardous to human health when it presents in high concentration in drinking water. One potential hazard of nitrification is that nitrite can react with secondary amines in soil to produce nitrosamines, which can be assimilated by plants and thought to be associated with cancer in humans (Killham 1994). Nitrogen may be lost in the atmosphere through denitrification by biochemical reduction reactions with the conversion of nitrate to gaseous forms such as nitric oxide, nitrous oxide and di-nitrogen gases. The organisms that carry out this denitrification are commonly present in large numbers and mostly heterotrophic anaerobic bacteria in genera, *Pseudomonas, Bacillus, Micrococcus* and *Achromobacter* (Brady and Well 2005).

A significant forest area in the tropics are presently undergoing the changes mainly due to anthropogenic reasons like human settlement, change in land use from forest to agriculture and substantial clearance of forest for collection of fuel wood, and over extraction of timber, as well as the lack of any management for other forest products and also through faulty management of forest such as clear felling of forests followed by artificial regeneration. Understanding these activities on long-term effects of soil properties and tree growth parameters is not clear in many cases (Staddon et al. 1998). One of the most practical approaches is to assess soil quality using microbial indicator, especially related to nutrient transformation and availability (Pankhurst et al. 1997). Since soil microorganisms are the driving force of nutrient supply in soils (Smith and Paul 1990), any kind of anthropogenic activity can lead to detrimental effect on the abundance, composition and activity of microorganisms. Soil microorganisms are also critical for the maintenance of below ground system, which in turn, essential for the sustainability of all terrestrial ecosystems (Neary et al. 1999). Soil characteristics supporting aboveground plant growth through maintaining soil structure, water-holding capacity, aeration, infiltration, nutrient status and many other such properties are largely related with the functioning of microbes. Therefore, the disruption of soil microbial community might have immediate and long-lasting deleterious effects on the ecosystem as a whole. Fire is one such force, which destroys soil physicochemical properties and disrupts soil microbial community including actinomycetes in the forest ecosystem. Actinomycetes of the genus Frankia can fix nitrogen in the root nodules of non-leguminous angiosperms such as *Alnus glutinosa* (alder). Actinomycetes are widely distributed in different types of soils but more abundant in surface soil. In dry soil with a high pH, their population is much higher than other microorganisms. In a recent publication on the effects of shifting cultivation in CHTs, many important roles of fungi and bacteria in soil are described (Miah et al. 2010).

Many studies on the processes, rates and several other aspects of ammonification, nitrification and denitrification were done from temperate forest ecosystem (Davidson and Swank 1987; Robertson et al. 1987; Schmidt et al. 1988; Groffman and Tiedje 1989; Killham 1990; Pankhurst et al. 1997; Haque et al. 1999; UNEP 2001; Zak and Grigal 1991). For example, Haque et al. (1999) reported afforestation effects on ammonification and nitrification rates on former agricultural soils in north east Scotland, UK. Similarly, nitrogen mineralizing, nitrification and denitrification rates in upland and wetland ecosystems were evaluated in east-central Minnesota, USA (Zak and Grigal 1991). In tropical region, specifically on population of nitrogen transforming microorganisms such as Azotobacter, ammonifying bacteria, Nitrosomonas, Nitrobacter and denitrifying bacteria including blue green algae were estimated under seven different introduced and indigenous tree species in the upland watershed of Bangladesh (Gafur 2001). Two different studies were done in the forests of Cox's Bazar, Bangladesh, on the effects of clear felling of natural forest followed by artificial regeneration to estimate destruction of forest and soil erosion from this silvicultural operation (Haque and Alam 1988; Haque and Osman 1990). The present study was undertaken to investigate effects of forests clear felling and subsequent burning on population of several soil micro-organisms, which do not bring in any consideration in managing forests in Bangladesh, although they play an important role as a decomposer within the soil as well as in maintaining ecological balance in the overall environment.

Materials and methods

Site description

Chittagong University campus covers 710 ha land, and lies between 22°27'30" N and 22°29'0" N latitudes and between 91°46'30" E and 91°47'45" E longitudes. The area enjoys a tropical monsoon climate. Mean annual rainfall is 273 cm, of which over 80% rainfall occurs between May and September, and mean monthly temperature varies from 19.44 °C in January to 28.88 °C in May (UNEP 2001). The hills are about 20 m in elevation and comprised of unconsolidated folded Tertiary rocks of the Dupitila formation and possess somewhat excessively drained soils with silt loam to clay loam textures, falling in Barkal soil series locally and in Typic Dystrochrepts of US soil taxonomy (Islam et al. 1979). Primary forest of the hills was cleared long ago and covered with secondary vegetation such as thickets with a few scattered trees, thatching grasses and bamboo. Now most of the areas are under artificially planted forest by the Institute of Forestry and Environmental Sciences and Department of Botany, under University of Chittagong. Three pair sites, each representing clear felling area and mixed plantation situated side by side with similar conditions edaphically, were selected in Chittagong University campus, Bangladesh, to investigate the effects of clear felling on soil properties. Clear felling was carried out in May 2007. Soil samplings from this area and adjacent mixed plantations were done four months after felling in September 2007. Burning was done on three sites of the clear felled area in June 2008, i.e. one year after clear felling. The size of each burning site was 1 m×1 m. Each site was burnt vigorously for one hour with dried leaves, straw, branches and green vegetation to bring equivalent burning intensity usually during land preparation

before replanting and after clear felling of previous tree crop at maturity in Bangladesh. Soil samplings were carried out on the same day after burning and from the adjacent mixed plantations. Each of the pair sites is described as follows:

(1) Clear felling area with adjacent 15 year old 1994 mixed plantation: This pair site consisted of 4 100 m^2 clear felling area carried out in 2007 and adjacent 15 year old mixed plantation of *Dipterocarpus turbinatus* (garjan), *Acacia auriculiformis* (akashmoni) and *Eucalyptus camaldulensis* (eucalyptus). This site was on a moderately steep hill of 18% slope. The clear felling area was on eastern aspect situated to the north side of garage in the Institute of Forestry and Environmental Sciences under Chittagong University (IFESCU), with geographical position 22°27'89" N and 91°47'79" E. This felled area was previously covered with 15 year old *A. auriculiformis* plantation, and during sampling the area had ground coverage of 40% consisting of scattered distribution of creeper, herbs and grasses. The adjacent mixed plantation consisted of *D. turbinatus*, *A. auriculiformis* and *E. camaldulensis* was 15 year old on southeastern aspect of a 25% hill slope. The canopy coverage of the mixed plantation was 75% with ground coverage of 35% comprised of grass and herbs, mainly of *Clerodendrum viscosum* (bhat) with little or no litter.

(2) Clear felling area with adjacent 17 year old 1992 mixed plantation: This pair site was consisted of 6 100 m^2 clear-felled area carried out in 2007 with adjacent 17 year old mixed plantation of *Chickrassia tebularis* (chickrassi) and *Lagerstroemia speciosa* (jarul). Geographical position of this site was 22°27'94" N and 91°47'77" E on eastern aspect of hill situated to the south of Paharika housing society, Chittagong University. The area was previously covered with 14 year old *A. auriculiformis* plantation on a 15% hill slope. Ground coverage of this clear felled area was 40% consisting of scattered creeper, grasses and herbs, mainly of *C. viscosum*. The adjacent mixed plantation of *C. tebularis* and *L. speciosa* was 17 year old on eastern aspect of a 20% hill slope. Canopy coverage of the plantation was 70% with 45% ground coverage, mainly of climbers, *C. viscosum* and grass with little or no litter.

(3) Clear felling area with adjacent 21 year old 1987 mixed plantation: This pair site was on the northeastern aspect situated to the west of the Nipobon School in Chittagong University campus located at 22°28'15" N and 91°47'61" E geographical position. This site was consisted of 8 100 m^2 clear felling area carried out in 2007 with adjacent 21 year old mixed plantation of *Swietenia macrophylla* (mahagoni) and *Albizia lebbeck* (koroi). The clear felling area was previously covered with 14 year old *A. auriculiformis* plantation on 24% hill slope. The ground coverage was 50% consisted of *C. viscosum*, *Eupatorium* spp. (asamlata) and grasses scatteredly. The adjacent mixed plantation was of 21 year old *S. macrophylla* and *A. lebbeck*. This hill of mixed plantation was similar to clear felling area with respect to slope and aspect. Canopy coverage of the area was 75% with ground coverage of 80%, mainly of *C. viscosum* with climbers, grasses and herbs with 2 cm litter depth.

Soil sampling and analysis

From each of the above mentioned paired sites five replicated soil samples were collected from a depth of 0−5 cm, mixed thoroughly to give a composite sample and brought to the laboratory in labeled polybag, sterilized with 95% ethyle alcohol. In the laboratory, samples were divided into two sub-soil samples: one used for the determination of physico-chemical properties and the other kept in the incubator at 4 °C temperature for determining biological properties.

Soil physico-chemical properties

Moisture content was determined after drying the soil in an oven at 105 °C for 8 h and pH of moist soil at 1:2 soil-water ratios by TOA pH meter in triplicate. Soil organic matter was determined by loss on ignition method according to Ball (1964).

Microbial population

For isolation, 1 g of soil was taken with 99 mL of sterile water in a conical flask to prepare 1:100 dilution and shaken vigorously for 30 times. From this suspension 1 mL was taken out using sterilized pipette and mixed thoroughly adding 99 mL sterile water in another conical flask to give 1:10 000 dilution. In this way, soil suspension was diluted up to $1:10^9$. Dilutions $1:10^4$, $1:10^5$, $1:10^6$ and $1:10^7$ were used for culturing, isolation, and estimation of actionmycetes and other four types of soil organisms, *viz*. *Rhizobium* and ammonifying, nitrifying and denitrifying bacteria.

Rhizobium: For estimation of *Rhizobium* population, yeast extract mannital agar (YEMA) medium was used (Clark 1965c). In preparation of YEMA medium, 10 g mannital, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 g NaCl and 0.1 g yeast extract were taken in a beaker and 1 000 mL distilled water added. Exactly, 20 g agar powder was added gradually to the solution with gentle heating and constant stirring with a glass rod. The content was then boiled for 15 minutes, transferred into two 500 mL conical flasks, autoclaved and plated. Total count of *Rhizobium* was done by spread plate method, in which 15−20 mL YEMA medium poured in 15 petri plates and allowed to solidify. Then 1 mL of soil suspension was pipette out to petri plates from each of the dilutions of $1:10^2$ to $1:10^6$ sequentially with 3 replications. Then, the plates were incubated at 27 °C. After 48 h, all the perti plates were examined for a particular dilution range. Petri-dishes which had >300 or <30 colonies or colony diameter greater than 2 cm in any plate were discarded (Clark 1965b). Total numbers of colonies on each of the accepted plates were counted by using colony counter. The result was expressed as Colony Forming Unit (cfu).

Ammonifying bacteria: For estimation of ammonifying bacteria nutrient broth solution was used, prepared from 5 g peptone and 3 g beef extract adding 1000 mL distilled water. Then the mixture in beaker was gently heated and stirred continuously. Four test tubes, each containing 7 mL sterile nutrient broths were inoculated with 1 mL, 0.1 mL, 0.01 mL and 0.001 mL soil sus-

pensions from $1:10^4$ to $1:10^{-7}$ dilutions, respectively. The inoculated tubes were then incubated at 30°C for 30 days in lamina flow. Then 3−5 drops of Nessler's reagent were prepared from potassium mercuric iodide solution and sodium hydroxide and added to each test tube. Development of brick red color in the test tube indicated the presence of ammonifying bacteria. Most Probable Number (MPN) of ammonifying bacteria was calculated only for the positively inoculated test tubes using MPN chart (Alexander 1965b).

Nitrosomonas nitrifying bacteria: Ammonium calcium carbonate medium was used for culturing *Nitrosomonas* nitrifying bacteria (Alexander and Clark 1965). This medium was prepared taking 0.5 g $(NH4)_2SO_4$, 1 g K_2HPO_4, 0.03 g $FeSO_4{\cdot}7H_2O$, 0.3 g NaCl, 0.3 g $MgSO_4{\cdot}7H_2O$ and 7.5g $CaCO_3$ in 1 000 mL beaker and adding distilled water up to the mark. The contents were then warmed and stirred continuously. Four test tubes, each containing 7 mL ammonium-calcium carbonate was inoculated with 1 mL, 0.1 mL, 0.01 mL and 0.001 mL soil suspension of dilutions from $1:10^4$ to $1:10^7$, respectively, by using lamina air flow and incubated at 30°C for 30 days. Then, 3 to 5 drops of Griess-Ilosvay reagent were added to each of the tubes. Development of purple-red color in inoculated tubes indicated presence of nitrite. Otherwise, a small pinch of the Zn-Cu-MnO_2 (1:1:1) mixture was added and development of reddish color indicated presence of *Nitrosomonas*. Zinc-copper-manganese dioxide was prepared with mixing powder of 1 g Zn, 1 g MnO_2 and 1 g Cu together and kept in a dark bottle for use during the test (Alexander and Clark 1965). MPN of *Nitrosomonas* was calculated using the chart only for positive tubes. Griess-Ilosvay reagent was prepared by mixing three different solutions together mentioned hereafter. The first solution was prepared by dissolving 0.6 g sulfanilic acid in 70 mL hot distilled water and adding 20 mL concentrated HCl. The final volume of the solution was made 100 mL with distilled water. The second solution was prepared from 0.6 g alpha-nahpthylamine and 1 mL concentrated HCl. The solution volume was then made to 100 mL with distilled water. The third solution was prepared dissolving 16.4 g $CH_3COONa.3H_2O$ in distilled water and making volume to 100 mL with distilled water. Thus, three prepared solutions were stored separately in dark bottles and kept in a refrigerator. Three solutions were mixed together in equal proportions just prior to the test (Alexander and Clark 1965).

Nitrobacter nitrifying bacteria: Nitrate-calcium carbonate medium was used for culturing *Nitrobacter* (Alexander and Clark 1965). This medium was prepared from 0.5 g KNO_2, 1 g K_2HPO_4, 0.03 g $FeSO_4{\cdot}7H_2O$, 0.3 g NaCl, 0.1 g $MgSO_4{\cdot}7H_2O$, 7.5 g $CaCO_3$ and 0.3 g $CaCl_2$, and added by distilled water up to the mark in a 1 000 mL volumetric flask. The content was warmed, stirred continuously and autoclaved successively. Nitrite-calcium carbonate medium was then inoculated with same dilutions of soil suspension as above. After incubation, 3−5 drops of Griess-Ilosvay reagent were added to each tube. Colorless solution in inoculated tubes indicated the presence of *Nitrobacter*, and MPN was calculated as for the said organism (Alexander and Clark 1965).

Denitrifying bacteria: Two different solutions mixing together were used as medium for culturing denitrifying bacteria. The first solution was prepared from 1 g KNO_3, 1 g asparagine and 5.0 mL bromothymol blue with 500 mL distilled water. The second solution was prepared from 8.5 g Na citrate, 1 g KH_2PO_4, 1 g $MgSO_4{\cdot}7H_2O$, 0.2g $CaCl_2{\cdot}6H_2O$ and 0.05g $FeCl_3{\cdot}6H_2O$ with 500 mL distilled water. After preparation of first and second solutions, both were mixed together adjusting pH between 7.0 and 7.2, which showed green color. This mixed medium was then inoculated with same dilutions of soil suspension as above. Vigorous gassing and blue coloration indicated the presence of denitrifying bacteria (Alexander 1965a) and MPN was then calculated as for said organism.

Actinomycetes: Oat meal agar (OMA) medium was used for culturing actinomycetes. This medium was prepared from boiling 20 g oatmeal in sufficient water to give 1 000 mL extract. After boiling the content was filtered and made the volume to 1 liter. Thus, the extract collected was heated on a gas stove. Then 15 g agar powder was added to the extract and stirred constantly to prevent scorching the bottom of the pan and to dissolve agar fully. All glassware was washed thoroughly with distilled water. The prepared medium was then poured carefully to 3 or 4 conical flasks and plugged with cotton in the mouth. The agar media has a tendency to form ball suddenly, so to avoid ball forming the flask media should not be kept for sufficient time without heating. Each of the OMA media in conical flask was carefully unwrapped. Cotton plug was then removed from the mouth of conical flask. Cotton adhered in the mouth of the conical flask was burnt in a spirit lamp. The lid of the plates was opened at 45° or as required. About 15 mL of sterilized OMA media from conical flask was poured in Petri-dish and rotated clockwise and anticlockwise several times to spread the media evenly throughout the petridish. In this way 18 different replicated petri-dishes were prepared from each of the media. Exactly, 0.8 mL lactic acid solution was then added with 500 mL media in a conical flux and spread to petri-dish for inhibiting fungal growth. For isolation of actinomycetes, 1 mL soil dilution was taken from $1:10^4$, $1:10^5$, $1:10^6$ and $1:10^7$ dilutions. For actinomycetes, after 7–10 days incubation, all petri-dishes were examined whether a particular dilution range plated and an exact dilution effect achieved or not. A plate prepared from a given dilution should have only one tenth of colonies of the plate prepared from the next lower dilution. Petri dishes were discarded which had numerous colonies on the plates and had no dilution effect. Plates with colonies between >25 and <300 with satisfactory appearance were selected from the dilution (Clark 1965a). Total number of colonies on each of the suitable plates was enumerated using colony counter.

Statistical analysis

Each of the soil properties for triplicate sub-soils from a composite sample were tested to compare the means between the two treatments – mixed plantation and clear felled area or burnt area through one way ANOVA by SPSS program to find out the significance level.

Result and Discussion

Soil physico-chemical properties

At all the three pair sites of Chittagong University campus, clear felling area or burnt site showed significantly ($p \leq 0.05$ to $p \leq 0.001$) lower moisture and organic matter, and significantly ($p \leq 0.05$) higher pH compared to adjacent 15–21 year mixed plantation, with one exception for moisture (Table 1 and 2). At the end part of rainy season, i.e. in September, mean values of moisture, organic matter and pH in clear felling area were 30.31%, 6.44% and 5.74, while in adjacent mixed plantations the values were 34.14%, 11.04% and 5.11, respectively (Table 1). Mean values of soil moisture, organic matter and pH in burnt area were 6.93%, 6.05% and 6.18, and in adjacent mixed plantations the values were 15.27%, 9.82% and 5.04, respectively, at the start of rainy season, i.e. in June (Table 2). Moisture contents were also variable between the two sampling periods in mixed plantations being much higher in September and much lower in June (Table 1 and 2). In different aged mixed plantations moisture contents varied from 27.55% to 39.55% in September and from 13% to 19% in June. However, contents of organic matter did not show any definite trend between the two seasons and in mixed plantations organic matter contents varied from 7.22% to 15.03% in September and 9.82% to 11% in June (Table 1 and 2). Soil pH significantly increased and moisture significantly reduced due to burning at the present location**.**

Table 1. Soil physico-chemical properties in clear felled area and adjacent forest land in September 2007 at Chittagong University campus, Bangladesh

Silvicultural treatment	Moisture content (%)	Organic matter (%)	pH
15 year mixed plantation	[a] 35.31*	15.03***	5.06*
Adjacent clear felled area	31.66*	6.06***	5.63*
17 year mixed plantation	27.55*	10.87***	5.16*
Adjacent clear felled area	29.16*	7.31***	5.73*
21 year mixed plantation	39.55***	7.22***	5.12*
Adjacent clear felled area	30.12***	5.95***	5.86*
Mean of mixed plantation	34.14	11.04	5.11
Mean of adjacent clear felled area	30.31	6.44	5.74

[a] indicates that each value is the mean of three sub-soil samples of a composite of five samples from the field; * indicates significant difference between the means of each silvicultural treatment at $p \leq 0.05$ and *** indicates at $p \leq 0.001$.

Soil biological properties

At Chittagong University campus population of bacteria such as *Rhizobium*, ammonifying bacteria, *Nitrosomonas*, *Nitrobacter* and denitrifying bacteria and actinomycetes were very significantly ($p \leq 0.001$) lower either in adjacent clear felling area or burnt soil than in mixed plantations at all the three pair sites (Table 3 and 4). In other word, both clear felling and burning drastically reduced bacterial and actinomycetes population in soil, for which lower moisture content and higher pH in soil might be the one of the reasons (Table 1). Particularly, moisture content as well as pH of soil influence abundance of the organisms. In the present study also abundance of all kinds of organisms in plantations, clear felling areas and burnt sites were much higher at the end of rainy season than at the start of rainy season suggesting optimum moisture content in soil favors growth of microorganisms in soil. Microbial population is sensitive to moisture condition and with the fluctuations of moisture both community-size and composition change in soil (Pritchett and Fisher 1987). Moisture affects microbial growth through availability of oxygen in soil, which at too high condition restrict microbial growth. For example, the number of actinomycetes in 15, 17 and 21 year plantations in September were 123.67, 136.93 and 107.53 million cfu·g^{-1} oven dry soil, respectively, and their corresponding values in June of the next year were 111.32, 121.11 and 110.47 million cfu·g^{-1} oven dry soil. *Rhizobium* population in adjacent clear felling area was 8.30 ×10^4 cfu·g^{-1} oven dry soil, while in 21 year mixed plantation 105.87 ×10^4 cfu·g^{-1} oven dry soil. When this clear felling area was burnt population of *Rhizobium* became 0.57 ×10^4 cfu·g^{-1} oven dry soil and 21 year mixed plantation showed 95.15×10^4 cfu·g^{-1} oven dry soil. Actinomycetes population in adjacent clear felled area was 52.45 million cfu·g^{-1} oven dry soil and in 17 year mixed plantation 136.93 million cfu·g^{-1} oven dry soil (Table 3). In this clear felling area after burning actinomycetes population were 25 million cfu·g^{-1} oven dry soil and in 17 year mixed plantation had 121.11 million cfu·g^{-1} oven dry soil (Table 4). Population of *Rhizobium*, ammonifying bacteria, *Nitrosomonas*, *Nitrobacter* and denitrifying bacteria and actinomycetes in three clear felled areas per gram oven dry soil on an average were 5.82×10^4 cfu, 1.02×10^7 MPN, 0.81×10^7 MPN, 0.65 ×10^7 MPN, 0.76×10^7 MPN, and 58.92 million cfu, respectively. Corresponding values of these organisms in mixed plantation in each gram of oven dry soil were 73.11×10^4 cfu, 2.22×10^7 MPN, 1.67×10^7 MPN, 1.67 ×10^7 MPN, 1.57×10^7 MPN and 122.71 million cfu. Population of *Rhizobium* in burnt soil was 0.33 ×10^{4}cfu, ammonifying bacteria 0.42×10^7 MPN and actinomycetes 28.14 million cfu·g^{-1} of oven dry soil, while their corresponding values in mixed plantations were 61.76 ×10^4 cfu, 1.81×10^7 MPN and 110.47 million cfu·g^{-1} of oven dry soil. Population of *Nitrosomonas*, *Nitrobacter* and denitrifying bacteria was same (0.03 MPN·g^{-1} oven dry soil ×10^7) in burnt soil and values of these three different organisms in mixed plantations were 1.59, 1.39 and 1.44 MPN·g^{-1} oven dry soil ×10^7, respectively. All these findings for different soil microorganisms were in agreement with other researchers from home and abroad. Population of both types of nitrifying bacteria-*Nitrosomonas* and *Nitrobacter*, and denitrifying bacteria were also lower either in clear felling area or burnt forest land than in different sites under 5 to 10 year old *A. auriculiformis* plantation in the same area of Chittagong University campus reported by Gafur (2001). Here he found in per gram oven dry soil *Nitrosomonas*, *Nitrobacter* and denitrifying bacteria 1.21, 1.21 and 1.32 MPN×10^6, respectively.

Population of ammonifying, nitrifying, denitrifying bacteria in the burnt area at present studied sites were also lower compared to varied aged (4.35 year) mono-plantations at 59 different sites in hilly region of Bangladesh reported by Gafur (2001), indicating burning definitely reduce soil microbial population. Outside the country, Rao et al. (1989) found increased microbial population including nitrifying bacteria in soils at 0–15 cm depth under 22-year-old plantations of 10 different tree species in a research farm of Rajasthan in India, the neighboring country, under similar climatic condition, compared to barren land. Nitrifying bacteria were also found more numerous in *Pinus elliottii* stands than in burned stand of *E. micrantha* forest (Jones and Richards 1977). Several other authors (Bissett and Parkinson 1980; Beschta et al. 2004) reported that pattern of response in total micro flora was associated with an immediate decrease in amount of microbes after burning, which supported present finding of lower population in several types of nitrogen transforming organisms in burnt soil compared to soil under mixed plantations at all the three locations. In a separate study, in several places of CHTs showed that soil microorganisms such as fungal and bacterial population in surface and subsurface soils was significantly ($p \leq 0.05$) lower in shifting cultivated land, which involves burning during land preparation, compared to village common forest as better representation of natural forest (Miah et al. 2010). All these reports from home and abroad as well as findings of the present study, therefore, suggest that clearing or absence of forests and burning in no way is beneficial to microbial population in soil.

Table 2. Soil physico-chemical properties in burnt and adjacent forest lands in June 2008 at Chittagong University campus, Bangladesh

Silvicultural treatment	Moisture content (%)	Organic matter (%)	pH
15 year mixed plantation	[a]19.00***	11.00***	5.00*
Adjacent burnt site	7.00***	4.47***	5.91*
17 year mixed plantation	13.80***	8.94*	5.10*
Adjacent burnt site	5.80***	7.87*	6.36*
21 year mixed plantation	13.00***	9.52*	5.02*
Adjacent burnt site	8.00***	5.81*	6.28*
Mean of mixed plantation	15.27	9.82	5.04
Mean of adjacent burnt site	6.93	6.05	6.18

"a" indicates that each value is the mean of three sub soil samples of a composite of five samples from the field; * indicates significant difference between the means of each silvicultural treatment at $p \leq 0.05$ and *** indicate at $p \leq 0.001$.

To conduct an experiment for long term is quiet absurd in Bangladesh after clear felling a forest area with subsequent burning of land taking permanent sample plots. Here, plantations at maturity are felled by the Bangladesh Forest Department as their own plan and program, and replant within 6 to 12 months after clearing and burning of all debris. Institute of Forestry and Environmental Sciences under Chittagong University (IFESCU) also has been planting tree seedlings outside normal academic program since inception of the institute in 1977. During this period upon reaching three plantations of *A. auriculiformis* at maturity situated adjacent to 15–21 year mixed plantations at three locations were felled, each covering very small area ranging between 4 100 m^2 and 8 100 m^2, and replanted within one year of felling. As such, the area being situated near IFESCU on low hills of medium to gentle slopes provided a good opportunity to conduct this study. Within one year of felling, the area replanted again with new seedlings of local species. Hills under this research were not free from biotic and management based factors. The gap period was only four months between felling of plantations and soil sampling. Even then differences were distinct in soil microbial population within this short period due to forests felling. The usual procedure of reforestation practiced by Forest Department in Bangladesh is through clear felling of forests, both natural and manmade one, the remaining vegetation on land burnt after slashing and drying. In the IFESCU campus, the felled forest areas was not burnt as a means of land preparation, but slashed again with the presence of excessive vegetation of herbs and shrubs in places. Therefore, in the present experiment, to match with usual procedure of reforestation, patches of lands were burnt at intensity similar to practiced by the Forest Department for the last long years for preparation of land to re-afforest the felled areas. Soil sampling was done purposively from 0–5 cm soil depth with the aim of getting maximum effect of this silvicultural treatment on microbial population, since surface soil became the home for most of the soil inhabitants receiving more light, air and organic matter source.

Abundance of nitrogen transforming bacteria and actinomycetes are very important in maintaining soil fertility and natural ecosystem through processes like ammonification, nitrification, denitrification and nitrogen fixation. Drastic reduction in their population through clear felling of forest and subsequent burning of debris from the population with the presence of established forest would certainly create several environmental consequences and nutritional problems in plants. Reduction in nitrifying bacteria would hamper in supplying of nitrogen in the form of NH_4^+ and NO_3^-. Again, with the reduction of denitrifying bacteria removal of excess nitrogen from applied fertilizer would increase leaching loss of NO_3- through the soil profile into drainage water. Leaching of NO_3- is much higher in Bangladesh due to high rainfall and application of high amount of nitrogenous fertilizer, and this has also both considerable economic loss and environmental concern such as killing of aquatic animals and the hazard to human health in excess concentration in drinking water. Similarly, drastic reduction in soil microbial population through clear felling of forest with subsequent burning as a reforestation method might be one of the causes for green house effect, ozone layer depletion and global warming, when nitrous oxide becomes the end product of denitrification (Crutzen and Ehhalt 1977; Wang et al. 1976). Denitrification also acts as a "safety-valve" by releasing nitrogen from soils that receive heavy pollutant loads of nitrogen from acid rain (Killham 1994). On the other hand, nitric oxide and nitrous oxide release to the atmosphere by denitrification form nitric acid, which is one of the principle components of acid rain. Nitrogen oxide gases reacting with volatile organic pollutant form ground level ozone and a major air pollutant in the photochemical smog. Nitric oxide rising to the upper

atmosphere contributes to the greenhouse effect absorbing infrared radiation, as much as 300 times that of equal amount of CO_2 and nitrous oxide moving up into the stratosphere, and reactions result destruction of O_3 and becomes one of the causes of additional skin cancer. Thus, many biochemical processes accomplished by soil organisms are related with performing of many natural ecosystem of the environment.

Table 3. Nitrogen transforming bacteria and actinomycetes population in clear felled and adjacent forest lands in September 2007 at Chittagong University campus, Bangladesh

Silvicultural treatment	*Rhizobium* (cfu·g^{-1}oven dry soil ×10^4)	Ammonifying Bacteria (MPN·g^{-1}oven dry soil ×10^7)	Nitrifying bacteria (MPN·g^{-1} oven dry soil ×10^7)		Denitrifying bacteria (MPN·g^{-1} oven dry soil ×10^7)	Actinomycetes (million cfu·g^{-1} oven dry soil)
			Nitrosomonas	*Nitrobacter*		
15 year mixed plantation	[a]95.99***	2.00***	1.60***	1.90***	1.40***	123.67***
Adjacent clear felled area	7.04***	1.50***	1.10***	0.92***	0.94***	63.15***
17 year mixed plantation	17.47***	1.90***	1.50***	1.50***	1.30***	136.93***
Adjacent clear felled area	2.13***	0.62***	0.60***	0.30***	0.61***	52.45***
21 year mixed plantation	105.87***	2.70***	1.90***	1.60***	2.00***	107.53***
Adjacent clear felled area	8.30***	0.94***	0.72***	0.72***	0.74***	61.15***
Mean of mixed plantations	73.11	2.20	1.67	1.67	1.57	122.71
Mean of clear felled areas	5.82	1.02	0.81	0.65	0.76	58.92

MPN means Most Probable Number; cfu means colony forming unit; "a" indicates that each value is the mean of three sub soil samples of a composite of five samples from the field; * indicates significant difference between the means of each silvicultural treatment at $p \leq 0.05$ and *** indicate at $p \leq 0.001$.

Table 4. Nitrogen transforming bacteria and actinomycetes population in burnt and adjacent forest lands in June 2008 at Chittagong University campus, Bangladesh

Silvicultural treatment	*Rhizobium* (cfu·g^{-1}oven dry soil ×10^4)	Ammonifying bacteria (MPN·g^{-1}oven dry soil ×10^7)	Nitrifying bacteria (MPN·g^{-1} oven dry soil ×10^7)		Denitrifying bacteria (MPN·g^{-1} oven dry soil ×10^7)	Actinomycetes (million cfu·g^{-1} oven dry soil)
			Nitrosomonas	*Nitrobacter*		
15 year mixed plantation	[a]75.25***	1.65***	1.55***	1.57***	1.35***	111.32***
Adjacent burnt site	0.41***	0.04***	0.03***	0.03***	0.03***	33.87***
17 year mixed plantation	14.93***	1.68***	1.49***	1.19***	1.23***	121.11**
Adjacent burnt site	0.01***	0.03***	0.03***	0.03***	0.03***	25.00***
21 year mixed plantation	95.15***	2.10***	1.72***	1.42***	1.73***	98.99***
Adjacent burnt site	0.57***	0.06***	0.04***	0.03***	0.03***	25.54***
Mean of mixed plantations	61.75	1.81	1.59	1.39	1.44	110.47
Mean of burnt site	0.33	0.42	0.03	0.03	0.03	28.14

MPN means Most Probable Number; cfu means colony forming unit; "a" indicates that each value is the mean of three sub soil samples of a composite of five samples from the field; * indicates significant difference between the means of each silvicultural treatment at p≤0.05 and *** indicate at p≤0.001.

Conclusion

Clear felling of forests followed by artificial regeneration and associated burning has drastic deteriorating effects on soil actinomycetes and nitrogen transforming bacterial population as well as other varieties of bacteria and fungal population ((Miah et al. 2010). Further study is needed in this tropical region to understand more impacts of this silvicultural practice on the myriad of organisms, which play many important beneficial roles in the soil environment. Clearing or absence of forests and burning in no way is beneficial to soil microbial population and create several environmental consequences. Therefore, ecological aspect of soil maintained by microorganisms must be taken into consideration in managing artificially planted forest, particularly in Bangladesh, in response to face existing environmental stress of the world.

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